Figure 1. Overlap-extension-PCR fragment with purD deletion

Overlap-extension-PCR fragment with recA deletion

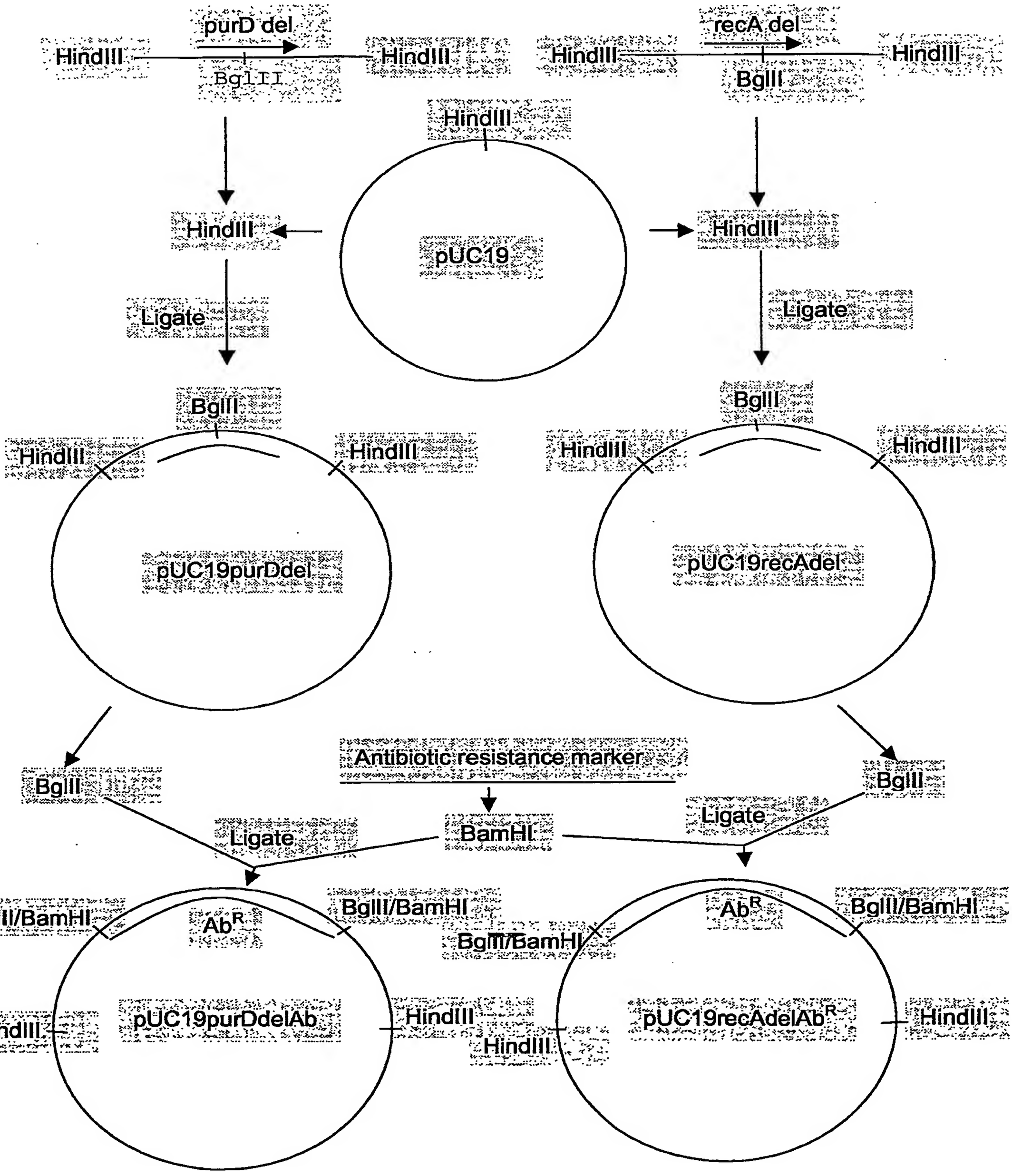

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Figure 2A.

```
   1  GTTCGACCAA ACGGCTTGTT GTGCGGTGAA ACATAGCACT CCTTGTGGCG TGGCTTTAGA TGATGATATT TTGCAAGCGT
      >>.......F5........>>          CTTAAGCTTGGA>>.......F13........>>
                                       ------
                                      HindIII

  81  ACCAAAAAGC ACACGACTGC GACCCGATTT CGATTTTTGG TGGCATTGTA ACTTTTAATA AAAAAGTAAC AAAAGCAGTG

 161  GCAGAAAAAT GTAACGAGAT TTTCCTTGAA ATCGTTGCTG CACCGAGCTT TGAGCCAGAG GCTTTGGAAG TTTTTGCTAA

 241  AAAGAAAAAT TTGCGCGTGA TTGAAGTTAA AAATCCATTA AGCGATAAAA TGCAACTCGT GCAAGTAGAT GGCGGATTGC

 321  TCGTGCAAGA AATCGACAAA TCGTTTAGCA ATGATTTTAA AGTAGTAACC GAAAAACAAC CTACCGAAAA GCAACTTTCT

 401  GATTTGGAAT TTGCCATGAA AGTAGTGAAA CATGTAAAGA GCAATGCCAT CGTGGTTGCC ACAAACGGAC AAGCTCTAGG

 481  CGTGGGCACA GGCGAGACTA ATCGTATTTG GGCAGCACAG CAGGCGATTC AGCGTGCAAA GGAAAAAACA CAAGAAAATC

 561  TAGTTTTGGC TTCCGATGCC TTTTTCCCAT TCAGAGATGT GGTAGATTAT GCAGCACAAG AAGGCATTAC AGCCTTGATT

 641  CACCCAGGAG GAAGCATGCG CGACCAAGAG AGCATAGACG CGGCTAATGA ACACGGAATC CCGATGATCA TCAGCGGTAT

 721  GAGACATTTC TTACATTAAA TCAAAAAATC TAAACAATAA TTATCAATAA TTCTAAAACA CAATAAGTAT GAATGCAAAT
                                                                          >>...purD...>

 801  GATTACAAAA AAATACTCAT CGTAGGAAAC GGCGCAAGAG AACACGCCAT CGGGTGGAAA ATTAAACAAG ACCACCCTTC
      >.....................................purD.....................................>

 881  TTGCGAGCTT TTCTTTGCGC CAGGAAACGC TGGAACCGAA CAAATTGGAA AAAACATCGT AGCTGAATCT AATTATGGCT
      >.....................................purD.....................................>
                          <<.........OE-R..........<<AGATCTGGCGCTACGCTAGAAG
                                                    -------
                                                    BglII

 961  TAATGCTTTT TGCTCAACAA AATGATATAG ACTTAACGAT TGTAGGTCCA GAAGCAGAAT TGGTAGAAGG TATTGTAGAC
      >.....................................purD.....................................>

1041  TTGTTTGAAT CCAATCAATT AAGAATTTTT GGTCCAGATA AGCGTGCGGC TAAATTGGAA GGCAGCAAGG CTTTTGCCAA
      >.....................................purD.....................................>

1121  AGATTTTATG GAGAAATACG GCGTGCGCAC GGCTTTTGCC AAAAGTTTCA ACAATTTTGT AGACGCTAGA GATTATGTAA
      >.....................................purD.....................................>

1201  AAGAGCTCAC GCAATTCCCT ATCGTGATCA AAGCCAGTGG CTTGGCAGCA GGAAAAGGTG TGATCATCGT GCACNTACAA
      >.....................................purD.....................................>

1281  CTTGAAGCCG AAACTACTTT GCGCAAAATC ATGGAAGACA AAACCTTTGG CGAAGCAGGC AACGAGGTCG TAATCGAGGA
      >.....................................purD.....................................>

1361  ATACTTAAAA GGTGTGGAAG TTTCTGTGCT TTCTATCTTT AACCATAAAG AAATTAAAAC TTTCTTGCCT GTAAAAGACC
      >.....................................purD.....................................>

1441  ACAAGAAAAT CGGAAAAGGC GAAACAGGAC TCAACACGGG CGGAATGGGC GTAGTGGCTC CTAACCCGCA TTTTACCGAT
      >.....................................purD.....................................>

1521  GAGCACATGA AGGAGTTTGA GAAAAACATT TTGCTCCCAA CACAAAAAGG GCTCTTGGCA GAAAAAATGC ATTTTGCAGG
      >.....................................purD.....................................>

1601  CATTATTTTC TTTGGGCTTA TGATTACCGA GCATGGTATT TATCTATTGG AATACAACAT GCGATTTGGC GACCCAGAAA
      >.....................................purD.....................................>

1681  CCGAAGCACT TTGCCTTTG ATGGAGAATG ATTTAGTAGC CCTCATCGAT TCCGCAATAC ACCAGCAAGA CATTGAACTT
      >.....................................purD.....................................>

1761  AAATGGAAAA ACGAACATGC TTGCTGTGTA GTAATGGCGA GCGGTGGCTA CCCAGGCACT TACGAAACTG GTTTTGAAAT
      >.....................................purD.....................................>
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```
1841  CCGAGGATTG AACAAAGTTG ATGTTCCCGT ATTTATTGCA GGAGCCAGAG AAGAAAGTGG AAAAATCTAC ACCACAGGCG
      >.....purD.....>

1921  GGCGCGTGCT CAATGTGGTG GGAACTGGCG CTACGCTAGA AGAAGCCAGA AAAGTGGCTT ACGAAAATAT CCATAAAATC
      >.....purD.....>
                        GAGATCTGG>>.....OE-F.....>>
                         ------
                         BglII

2001  AATTTTGATT ATGAATATTA TCGCGAAGAC ATCGGGAAGA TATAATCTCG CTGATTTTTA ACCAAAACAT ATTTAAAAAC
      >.....purD.....>>

2081  GCTTTTGTTA CTTTTATAAA CAAAGGCGTT TTTCTATTTT TGTGCCACTA TAACATGATT TAACCCATGA AAAAAATACT

2161  AAAAATACTC ATTTTTCTAC TGCTCATTCC TTGGGTTTAT GCCCTGATTT TAATCTTTAT AAATCCACCT ATCACCATTA

2241  CACAGCTGAG CAATTTATCT TATGGTTTCT CCAGAACACA GCTCGCTTAT GATGAAATTC CGGCTAGTGC TAAATGGGCT

2321  GTAATTGCAG CAGAAGACCA GAATTTTGCC ATTCATAATG GCTTTGATTT TAAAGAAATT AAAACCGCCT ACGAGAAAAA

2401  CAAAGCGGGC AAGAAATTGC GTGGCGGGAG CACCCTTTCG CAACAAACTG CCAAAAATGT ATTTTTGTGG CAAGGGCGCA

2481  CTTGGATTAG AAAAGGATTG GAAACCTACT GCACCTTTAT CATCGAAACG CTGTGGAGCA AGGAGCGTAT TTTGCAAGTT

2561  TACCTCAACA ATGCCGAAAT GGGCAAAGGC GTTTATGGCA TAGAGGCAGC GGCGCAATAT TATTTTAAGA AAAACGCCTC

2641  ACAGCTCACG CCTACCGAGA CGGCACGCAT CATTGCCTGC CTGCCCAATC CCAAAAAATA CAATNTAAAC CCGCCAAGTG

2721  CCTACATCTC AAAACGCGGA CAATGGATTC TGCGCCAAGT GCGAAACTTG AAAGGCGATA GGGCTCTGAG CGAGATTGTG

2801  AACACGCCCT AACGCCTGCC TCAACTCTTT GCACACAGTT TACCAACTCT CTGCGAAGAG TTCACAAACT CTTCGCACAC

2881  ACTTCCCCAA GTCTTTGCAA AGAGTTGGGA GATACTTAGG CACAAAAAAA AGGAACCTCA TGAATAGAGG TTCCCTCTTC

2961  CTTAAAAGGA ATAAATAATA ATGTTTTTTA AGCTTTAGGC TTGGCTACTT TTTCAAAGCC TGCTGCCTTC ATGCTATCTA
                                      -------
                                      HindIII

3041  GGATACGCTT GCCTGGGCGG TAGTTTACGC CTACCTTTTT GATTAAGCCC GAATGAAAAT CTTTCTCTGT ATCTGCCGCT
                                                                        <<......R8......<

3121  CCACTGCTTA AAGTGGCATA GAGCGAGCCA AGCTTATCTA AACGAACGAT TTTGCCCGCT GCCAAGGCGT CTTGAATTAC
      <R8.<<AAGCTTAAG
            ------                    -------
            HindIII                   HindIII

3201  ATTCTCTAGC GCAATGATAA CGCCACGAAT ATCTGCCTCG CTGAGTGCCG AAAACTTCTC GATTTGCTTA ACGAGCTGGT

3281  CTATATCCAT TTCTCCATCG CTTGCCACCA CGGCATAGTA TTTTTGTGGC TCCCCTGGCT TGCTTGGGTT TCTACGCTGA

3361  ATTACATTGT ATTTTATGCT CATAATTACT CTATTTTTAA TAGCCTCCCG ATGGATATAA AGTTACGCTA CAATTAGGGT

3441  CTCCATAAGC AAATCTATAC CCCTCTCTTT CATATTCCCT TCTCATTCTT CTTGCTCCAT CTCTCAAGGC ATCCGCTCTA

3521  TTACTGCTAT ACCCCTCCTG AAGAAATGTG TCTGCACTTG AAGAAGAATA TGAAGAGCTA TGAGAATCGT GCAACATAGT

3601  CCAAGCTCCA TCTTGAGCTA TAACATTTGC ATGACATGTA ACACCTATAG TATAATAAAA TCTCCTAGGA GGTTGTGTTC

3681  CACCACCACC TCCAGAGCTA CTACTTTTTT TACATTGTCC ATTTTGGTTA GCATGATTTT GTCCGCCATC ACTTACTAAC

3761  TTCTTAGCTT CTGCTAAGGC TTTTTCTCTT GCTTTCTTTT CAGCATCTGC TTGGCTAATT CCACTCACTG CTGTAGCTGT

3841  CGCTTCTTTT TTATAGTTTA CCGAGGTTCC ATAATAGCCA CTACTACAAT TGTTTCTTGT AAAGTTTTTA TTAAAAGATT

3921  GAGTTTGTGT TGAGGTGTAC CCTCCGAAAC CTTTTACTTC TACAGTAAAG GTAGAACTCC CCATGCTTAC GGGGAAGGTG

4001  GCGATAGTAT ACGATTGCCC TGCCGGCATT TGTTTTACTT GATACACTCC ATCTCCTCCC ACTTCTATGC TTGCCGTTAA
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4081  ATTACCACTA CCGCTAAAAG AGCCTTCTGC TATTTTTAGT GTTAAATCAT TTATATCCCC TCCTTGTCCT TTTGCAGAAG

4161  CTTTTGTTAC ACTTACAGCA TCATAAGCTC CTTTTCCATT GGTATAAGGT ATTTATATGG CCAAAC
```

Figure 2B.

```
   1  TAAAGCTGTA AWTCGCTATA AACGCCCTTT AGGATAAAAT CTGCCATTTT TTGCAGTATT TTWATAGCTA AAATTTAGAA
                            >>.......FrecAOR1.......>>

  81  AACACCATCT CGAGTAAAGG AGCGTGTAGT GCTCGCCATC GTTGAGCGAT TGCCCACCCT CAATTGATTT GGGCGAATAC
            CTTAAGCTT>>.........F6.........>>
               ------
               HindIII

 161  TTGAAATAAA TGGCATCTTC TAGCGACACA TTTTGCGCAG AAATCATGCA AAAAGCCCCG CATAAAAAGC TGAATAAAAA

 241  WGCTAWTYTT CTTGTTTAAA AAAACTCATA AATTCCCCCA AATATAGAAA TATTCTGTGA AAAGTTGCAA TTTATTAACA
                                                                                  <<...<

 321  CTATGTGCTT GCTTTTAATG AAAAAAGTAG ATTATTTTTC CGAATCCGAA AGTTTATTTA CGCCCCATCC GATGCCTAGT
      <..FrecA-4...<<

 401  CCCMSCGATA GCCATGATTA ATACAAATAC AATTAAATCA WATTTTTCMC MTWWACCATA GCACAACACT TGCTAGCTCA

 481  ACGAGTACTA GAGTGGTAAA AAGGATTTTT TGACGATTAT TCATGATTTT ATTTTTCTCA AAGGTAAATA TTTTAAACCA

 561  TAATTTCACA AATCTTAAAA TCTATTTAAA TAATAGAGAA ACCAGAAAAA AATCGTATTT TTACGGAATG AATAAAATGT

 641  TACAAGTAGG CGATAAAATG CCCGATTTCA AAGGTGTAGA CCAATTTGGG AAGGAGCATT CATCTGCCGA TTTCAAAAAT

 721  CAGAAATTAG TCGTTTTTTT CTACCCAAAA GCCAGTACGC CAGGTTGCAC GGCAGAGGCT TGCAACATCA ACGATAATCT

 801  TGATGCGCTA AAAGCACAAG GCTACCAAGT GATAGGCGTG AGTGCAGATT CGGTAGAAAA ACAACGAAAA TTCAGTGATA

 881  AATACGATTT TAAATTCCCT GTGATTGCCG ATGTGGATAA GAAAATTATT GAAGCATTTG GCGTGTGGGG CGAAAAGAAA

 961  TTCATGGGTA AAACCTATGA CGGAATTCAT CGTACGACAT TCATTATTGA TGAAAACGGA GTGGTGGAGC GCGTGATAGA
                         >>.......F7.......>>
                                  ------
                                  EcoRI

1041  AAAAGTGAAA ACAAAAGATC ATACCAATCA AATTTTAAAT TCAGAAAAAT AAAAATATGA GCGAAATAGA CGAAGCGAAA
                                                                  >>.........recA..........>

1121  AGGAAAGCAC TCCAGCTAGT GCTTGATAAA ATGGACAAAA GCTATGGTAA AGGTGCCGTG ATGATGATGG GCGACAAAGC
      >.....................................recA.....................................>
                                                                  <<.........OER1.........<

1201  CATAGACGAA AATATTCCAG TAATCCCTAC GGGGTCTCTA GGTTTAGATT TAGCCTTGGG CGTGGGAGGG TATCCGCGCG
      >.....................................recA.....................................>
      <CGAGATCTCGTGCGTGCGGT
          ------
          BglII

1281  GTAGAATCGT GGAGATTTAC GGTCCAGAAT CTTCTGGTAA AACCACTTTG GCAATTCATG CCATTGCCGA AGCTCAAAAG
      >.....................................recA.....................................>

1361  TCTGGCGGAA TTGCAGCTTT CATCGATGCA GAGCACGCAT TTGATAGATA TTACGCAGAA AAATTAGGCG TAGATGTTGA
      >.....................................recA.....................................>

1441  GCATTTAATT ATCTCTCAGC CAGATAATGG GGAGCAAGCT TTAGAAATTG CCGATAACTT AATCCGTTCA GGTGCAATTG
      >.....................................recA.....................................>
                                  -------
                                  HindIII

1521  ATATTATTGT AATCGATTCG GTAGCGGCTT TAACGCCAAA GTCGGAAATC GACGGAGATA TGGGCGATTC CAAAATGGGA
      >.....................................recA.....................................>

1601  TTGCAAGCGC GTTTGATGTC TCAAGCCTTG AGAAAGCTCA CGGGAACTAT CAATAAAACC AAATGTACTG CTATTTTCAT
      >.....................................recA.....................................>
```

```
1681  CAACCAATTG AGAGAGAAAA TCGGTGTGAT GTTCGGTAGT CCAGAAACCA CAACGGGTGG TAATGCACTT AAATTCTATG
      >.....recA.....>

1761  CATCGGTGCG TCTAGACATT CGTCGTTCTA CTCAGATTAA AGATGGGAAC GATGTCATCG GAAACTTGAC TCGCGTAAAA
      >.....recA.....>

1841  GTAGTGAAAA ACAAAGTAGC TCCGCCATTC CGTAGTGCAG AATTCGACAT TATGTATGGC GAAGGAATCT CTAAAGCAGG
      >.....recA.....>
                                              -------
                                               EcoRI

1921  CGAGATTTTA GACATTGCTA CCGATTTAGA AATCGTGAAA AAAAGTGGCT CTTGGTATTC TTATGCAGAT ACTAAACTAG
      >.....recA.....>

2001  GACAAGGGCG AGATGCCGTG CGTGCGGTAT TGAAAGATAA TCCAGAATTA GCCGAAGAAT TAGAAGAGAA AATTAAAGAA
      >.....recA.....>
            CGAGATCT>>.....OEF1.....>>
            ------
            BglII

2081  GAATTAGAGA AAAAATAGAT TTTTTAGTTT TTTTAATTAA ACGAAAAATC CGTTCACTTT GTTGAACGGA TTTTTTTATG
      >.....recA.....>>

2161  CTTGAATGAA TTTATTTCCA ATGGATTGAA TAGCCATGCA CTTTTAAATC TTCGCTATCA TAAGTGATTT CTTTGTCGGT

2241  GTTGGGATAG CAAACTTTAA GTCCTGCGTA TTTGGCAATG GCATGTCCTG CGGCAATGTC CCAAAAGTTT ACAGGTCTAA

2321  AGCGGGTGTA CTCCGTAGCC CACCGATCGG CAATTAGCCC AAGTTTGATA ACGCTTCCCA TAGGCTTTGT GCGGAAAATT

2401  TCATGTTCGG ATTTAATTTT TTTGATGTAT TCCTCGGTGC CAGGATCCAT GTGGAATTTG CTACAAAGAA AAGTGTAATC

2481  TTCGGGCAAA TCCATGGTAG GAATTGGCTT GCTGTGTTTC ATCAATTGTT CAAAAAAATC CGATTTCAGA GCCATTTTGT

2561  GCAATTGTTG TTGAGTCCCG ATGAATTTAC GAGAAGGGCA TTTATCGCTA CCGAAATAGA ACAATCCAAG CGATGGGGCG

2641  TACAAAACTC CTAGCTTAGC CGTATTATTC TCAACTAAGC CTAGACACAC GCAATATTCA TCTGTTTTGT TGACAAAATC

2721  CATGGTGCCA TCAATAGGGT CTGCAATCCA ATAGGTGGGC GTATTTCTAA TTTCTTGTAA AGAATCCTTA TCTCCTTCCT

2801  CACTAAAGTA TGGAATGTCT GTAAAGGAAA CATGTTTTTG CAAGATTTTG TTGGCGGCTA AATCTGCACT TGTAACAGGC

2881  GATCCGTCGG CTTTGGTCTC GGTGGAGAAT CCGTTTTGGA TTGTTTTAAA ACCTCTTCGC CAGCAAGTGC TACAGCCCGT

2961  GTTGCGATTT CTAATAAATT CATAATCATT CTTTTATTCT CGAACAAAGT CAAATAATTC TCTGTATTAA AAAATAATTT

3041  TGGCGATAAA AATTAAAATT TATATATAAA ATATCTCTGC AAAAAACCAA ATCAAATATT TAGTGAAATA AAAAAAATTA

3121  GATTGTAAAT TTGCCTTATG TTTTTAGAGA ATACCATAAA TCATAGAAAA AATACGGGCT GGATCGAAGT AATCTGTGGC

3201  TCTATGTTTT CGGGCAAAAC CGAAGAGTTG ATTCGTAGAG TGAAACGAGC CGAATTGGCT GGGCAAAAGG TAGAAATCTT
                                  <<.....R5.....<<AAGCTTAAG
                                                  ------
                                                  HindIII

3281  TAAACCCGCA ATTGATAAAC GCTACGATGA GCAAGATGTG GTATCGCATG ATGAAAACAA AAAACAAGCA ACCCCGATTG

3361  AGGCGAGTTC TAACTTGCCC ATTTTAGCAA GCGATTGTGA TGTGGTGGGG ATAGATGAGG CTCAATTCTT TGACGAAGGA

3441  ATTGTTGAGG TGGCAAATCT TTTAGCTAAT TCGGGGAAAA GAATAATTAT TGCGGGATTA GACATGGATT TTAAAGGTCG
                                                  <<.....RrecAOR1.....<<

3521  TCCATTTGGT CCTATGCCAA ATTTAATGGC GGTAGCGGAA TATGTGACCA AAGTGCATGC AATCTGTGTG AAAACAGGGA
```

table 5

group	no. of chickens	Treatment: vaccination at day 1		Treatment: challenge at day 25	Treatment: challenge at day 31	Results: % of max airsac score at day 10 (safety)	Results: % of max airsac score at day 38 (efficay)
1	25	NDV	RecA aerosol	NDV	WT-OR aerosol	2.5	25[b]
2	25	NDV	PurD aerosol	NDV	WT-OR aerosol	7.5	23[b]
3	25	NDV	WT-OR aerosol	NDV	WT-OR aerosol	68	10[b]
4	25	NDV		NDV	WT-OR aerosol	0	47
5	25	NDV		NDV		0	2

[b] Significantly different ($p<0.05$) compared to the controls (group 11) using two-sided Mann-Whitney U test

table 6

group	no. of chickens	Treatment: vaccination at day 1		Treatment: day 30	Treatment: challenge day 35	Results: % reduction
1	15		PurD aerosol	NDV	WT-OR aerosol	no reduction
2	15	NDV	PurD aerosol	NDV	WT-OR aerosol	54%[b]
3	15	NDV		NDV	WT-OR aerosol	no reduction
4	15	MA5		NDV	WT-OR aerosol	no reduction
5	15	MA5	PurD aerosol	NDV	VT-OR aerosol	50%[b]

[b] Significantly different ($p<0.05$) compared to the controls (group 11) using two-sided Mann-Whitney U test